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Seed Health Testing of Rice. V. Seed-Borne Fungi Associated with Abnormal Seedlings of Rice

by

F. C. GUERRERO, S. B. MATHUR and PAUL NEERGAARD

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SEED HEALTH TESTING OF RICE V. SEED-BORNE FUNGI ASSOCIATED WITH ABNORMAL SEEDLINGS OF RICE

F. C. Guerrero, S. B. Mathur and Paul Neergaard

Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark

Introduction

Occurrence of abnormal seedlings is a common phenomenon in seed testing for germination. Seedlings possessing all the essential structures and capable of producing normal plants when grown under favourable conditions are considered as normal. Abnormal seedlings are those which are incapable of producing normal plants. The laboratory evaluation is carried out in accordance with the International Rules for Seed Testing (ISTA, 1966) and USDA/Agriculture Handbook No. 30 (1952). Some of the causes inducing abnormalities in seedlings are mechanical damage to seeds during harvesting and threshing, artificial drying, chemical seed treatments resulting in phytotoxic effects, or infection with seed-borne organisms. In general the role of microorganisms in causing abnormal seedlings has not been well understood in seed testing laboratories.

Andersen (1955) in her studies on Merion Kentucky Blue grass (*Poa pratensis*) found through tests on blotters that abnormal seedlings with tall shoots but without roots as well as those with very short roots, were associated with fungi such as *Stachybotrys*, *Chaetomium*, and *Botrytis cinerea*. Working on germination and health of radish seeds de Tempe (1958) found that removal of apparently normal seedlings too early from the blotter during the test did not allow complete evaluation of abnormalities due to infection by *Alternaria* spp. or threshing injury. Marshall (1962) found *Ascochyta* species associated with weak, decayed pea seedlings having stunted primary roots. During seed health testing of flax de Tempe (1963) observed that extensive decay of seedlings could be caused by *Alternaria linicola* or *Botrytis cinerea*. Seedlings infected with *Alternaria linicola* were occasionally stunted because of root invasion by the fungus. *Botrytis cinerea* caused rapid decay of young seedlings. In germination tests de Tempe (1964) found that *Fusarium* spp. on cereal seeds reduced the percentages of normal seedlings, the extent of damage depending upon when the seedlings were evaluated and upon the degree of infection of the individual

grains. HEWETT (1964) found that carrot seeds infected with *Alternaria porri* f. sp. *dauci* produced abnormal seedlings with severe rotting. In Austria, wheat seedlings developing from *Septoria nodorum* infected seeds were observed by KIETREIBER (1962) to have malformed coleoptiles with brown spots and swellings (knobs). Such seedlings would be counted as abnormal in germination tests without knowing the cause.

The present investigation was carried out to establish whether seed-borne fungi are responsible for abnormalities in rice seedlings, and whether germination tests and seed health tests can be combined. A combined test would be useful in providing more complete information regarding the planting value of a seed lot.

MATERIALS AND METHODS

Twelve rice seed samples received from India, South Korea, South Vietnam, Japan, Thailand, Philippines, and Egypt were used in this investigation.

Seed Health Tests

Samples were tested by using the 'blotter method' (International Rules for Seed Testing, 1966). Four hundred seeds of each sample were placed in petri dishes on three layers of moistened blotters at the rate of 25 seeds per dish and incubated in a controlled environment room at 28°C under alternating cycles of 12 hours near ultraviolet light (NUV) and 12 hours darkness for 8 days. Seeds were then examined under a stereobinocular microscope (WILD M5) for the presence of seed-borne fungi.

Germination Tests

a. *Top of paper* (TP)

Four rice samples were used. This test was similar to the blotter method except that the petri dishes were placed under complete darkness in a controlled room at 28°C. Seedling evaluation and examination for the presence of seed-borne fungi was done simultaneously. Since it was not possible to evaluate the seedlings within 8 days of incubation these were left for a further period of 4 to 7 days. To avoid desiccation a few drops of water were added to the blotters after 8 days.

b. *Between paper* (BP – commonly called 'rolled towel method') This test was carried out simultaneously with the blotter method. In all, four hundred seeds of each sample, in four replicates, were spaced on a double layer of moistened filter-paper sheets (size 25 × 18 cm) and

covered with a single sheet. The sheets were folded along one edge and then rolled up. The rolls were placed in polyethylene bags in a dark room at 28°C. After 8 days of incubation the seedlings were evaluated. Normal and abnormal seedlings were counted according to the International Rules for Seed Testing (1966). A second count of abnormal seedlings was made on completion of the test after 14 days. Abnormal seedlings were examined on both occasions for the presence of seed-borne fungi.

c. *Sand test*

Fifty seeds in 8 replicates were sown in plastic dishes (diameter 18 cm) with sand and moistened with tap water. The dishes were placed in a room at 28°C with light provided by cool fluorescent light tubes. Germination counts were made after 14 days.

In seed testing stations the quality of seed samples is judged first by the results of the purity test followed by germination.

In the International Rules of Seed Testing (ISTA, 1966) three substrates are specified for evaluating the germination capacity of rice. These are:

1. Between paper (BP)
2. Top of paper (TP)
3. Sand (S)

TABLE 1. Percentage germination and occurrence of abnormal seedlings in the rolled towel test and the sand test. (400 seeds tested per sample).

Institute's accession numbers of seed samples	Rolled Towel		Sand	
	Normal seedlings	Abnormal seedlings	Normal seedlings	Abnormal seedlings
1044	91	7	87	5
1083	78*	6	72*	1
1151	99	1	93	0
1155	98	1	94	1
1160	89	2	80	0
1546	96	1	93	0
1549	74*	13	59*	0
1550	93	4	76	1
1592	79*	14	60*	1
1796	92	3	84	0
1797	91	2	90	0
1965	95	3	85	1

* Germination below 80% and hence these samples will be rejected by seed analysts.

Experience gained by the senior author at the seed testing station of the Bureau of Plant Industry, Manila, suggests that the rolled towel test (BP) is preferable in testing rice for germination since it provides adequate moisture during the complete course of the test. For the same reason this method was employed during the present work. Germination figures obtained were compared with those in the sand test in order to have more reliable data on the planting value (Table 1). The blotter method as outlined above was conducted only to have information about fungi associated with the different seed samples (Table 2).

In the rolled towel method the evaluation was done on the 8th day. Only in some cases abnormal seedlings with growth and sporulation of fungi could be detected. Other abnormal seedlings having no mycelial growth and also those having only mycelium of a fungus, were transferred to the top of wet blotters in petri dishes and incubated at 20° (12 hours alternating cycles of NUV light and darkness). Generally 3 to 4 days were optimal for the fungi to sporulate.

Results

(1) *Abnormal seedlings*

The data on germination indicate that sample No. 1083 (Japan), 1549 (India) and 1592 (Philippines) had higher percentages of abnormal seedlings, and these seed lots would be rejected for export and even for local sowings.

The following abnormalities were observed and classified:

Abnormalities (Plates I & II).

Category 1. no root(s)
2. no root, decay in shoot
3. short root
4. decay in shoot (shoot usually weak and showing decay near the point of attachment to the grain)
5. decay in shoot and root
6. primary root showing browning, no branching or secondary development, usually shoot appearing weak
7. coiling or twisted shoot (shoot appearing weak and showing browning)
8. spindly, pale or watery shoot
9. weak, spindly primary root

The frequency of the different categories of abnormalities were observed in the following descending order – 5, 8, 2, 4, 6, 1, 9, 7, and 3.

Seedlings with decay in shoot and in root were encountered most frequently in

TABLE 2. Incidence of different pathogens recorded in seed samples using the standard blotter method at 28 °C.

Fungi	Institute's accession numbers of samples											
	1044	1083*	1151*	1155*	1160*	1546	1549*	1550*	1592*	1796*	1797*	1965*
Drechslera longirostrata	0	0	0.2	0	0	0.2	0	0	0	0	0	0
D. oryzae	9.5	9.5	8.2	13.2	16.5	1.0	47.7	12.0	30.7	1.5	0.8	16.0
D. rostrata	0	0	0	0	0.2	0.2	0	0.5	0	0	0	0
D. sativa	0	0.2	0	0	0	0	0	0	0	0	0	0
Fusarium dimerum	0	27.0	0	0	0	0	0	0	0	1.8	1.5	0
F. equiseti	0	2.5	0	0	0	0	0	0	0	0	0.2	0
F. graminearum	0	7.0	0	0	0.5	0	0	0	0	43.6	26.3	0
F. moniliforme	0	0	0.2	0	0.2	0	0	0	0	0	0.8	0
F. semitectum	0	2.2	0	0.2	0.8	0.8	0	0	0.5	0.5	0.2	23.5
Pyricularia oryzae	0	33.5	3.0	5.5	7.5	0	0	0	0	31.0	9.8	7.0
Trichoconis padwickii	18.5	1.0	25.0	52.0	47.2	0	69.2	7.0	30.5	13.0	6.8	39.0

*No tolerances fixed yet but if they were 10% for *D. oryzae*, *F. graminearum*, *P. oryzae* and 25% for *T. padwickii*, these 10 samples would be rejected on health grounds.

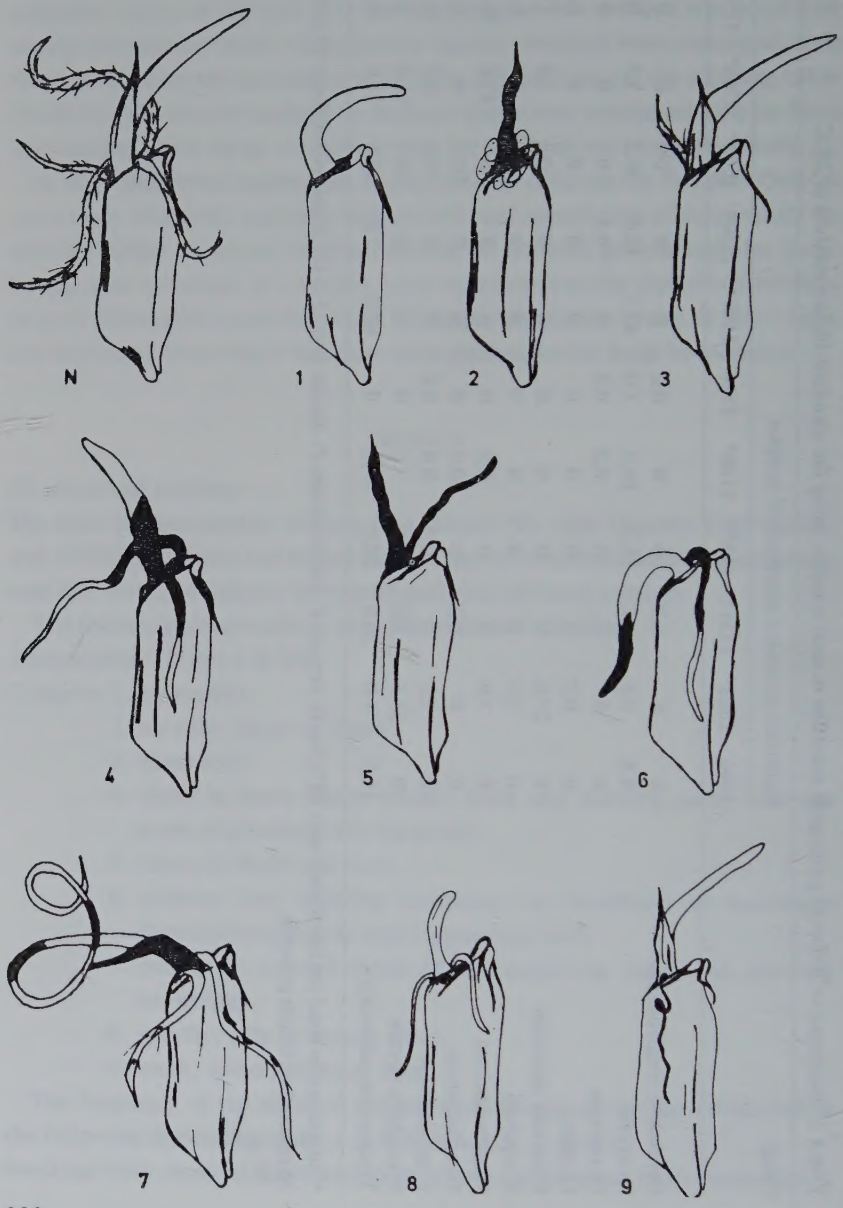
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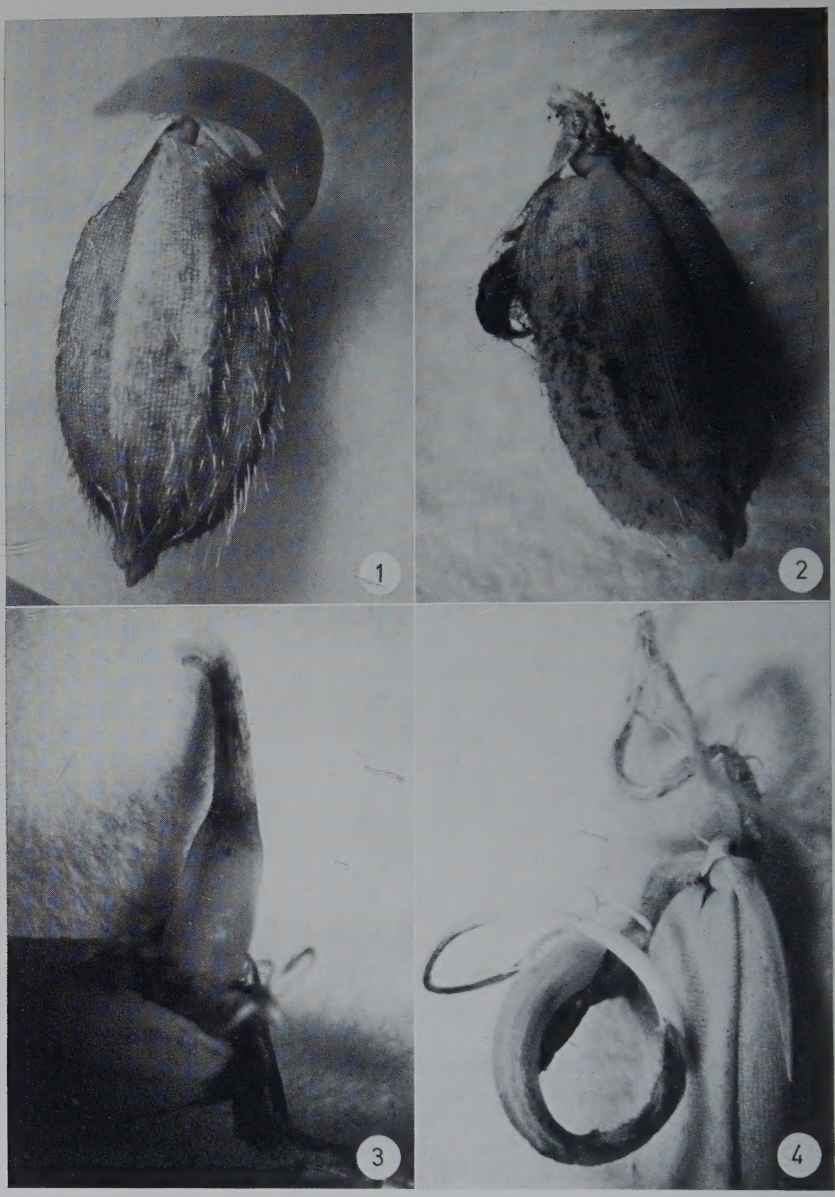
PLATE I. Diagrams of different categories of abnormal seedlings.

N – Normal seedling
1 – No root(s)
2 – No root, decay in shoot
3 – Short root
4 – Decay in shoot (shoot usually weak and showing decay near the point of atachment to the grain)
5 – Decay in shoot and root
6 – Primary root showing browning, no branching or secondary development, shoot usually appearing weak
7 – Coiling or twisted shoot (shoot appearing weak and showing browning)
8 – Spindly, pale or watery shoot
9 – Weak, spindly primary root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more than one half of the total abnormal seedlings (Table 3). The ones with watery shoots as well as those with no root but having decay in the shoot, were about 13%. Decay in the shoot of various orders and browning in the primary root occurred in 9% and 6% of the abnormal seedlings respectively. Other abnormalities were encountered in less than 1% of the seedlings.

Drechslera oryzae appeared on about 60% of the abnormal seedlings, *Trichoconis padwickii*, *Fusarium graminearum*, and *Pyricularia oryzae* in only 23%, 8% and 5% respectively. No fungus was found in 2% of the abnormal seedlings.

In category 5 (decay in shoot and root), *Drechslera oryzae* was found in 78% of the abnormal seedlings followed by *Trichoconis padwickii* (15%) and *Fusarium graminearum* (8%), *Pyricularia oryzae* occurred only in 2 abnormal seedlings.

(2) *Associated fungi and their role*

Fungi associated with different categories of abnormalities are listed in Table 4.

Drechslera oryzae (van Breda de Haan) Subramanian & Jain

Generally on the 8th day of incubation in the rolled towel method, mycelium and spores of the fungus were observed on decaying tissues of shoot and root of severely infected abnormal seedlings. This association indicated a direct relationship between the pathogen and the abnormalities (Table 4). The fungus was also found growing on the seed coat. In seedlings with mild necroses of shoot and root, the fungus in some cases sporulated only on the diseased portions. Most of the seedlings of this category had first to be transferred to the top of

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PLATE II. *Some of the abnormal seedlings*

1. An abnormal seedling having no root. Pale shoot covering the view of *Pyricularia oryzae* at the embryonal end.
2. Complete decay of shoot and root with heavy sporulation of fungi.
3. Pronounced decay in shoot and root. Mycelium with few conidia of *Drechslera oryzae* at the tip of the shoot.
4. An abnormal seedling with twisted, weak shoot and browning in root attacked by *Drechslera oryzae*.

TABLE 3. Distribution of abnormal seedlings in 12 rice samples.

Category of abnormal seedlings	Institute's accession numbers of seed samples												
	1044	1083	1151	1155	1160	1546	1549	1550	1592	1796	1797	1965	total
1. no root(s)		4								1			5
2. no root, decay in shoot		2					14	1	12		1		30
3. short root	1												1
4. decay in shoot (shoot usually appearing weak and showing decay near the point of attachment to the grain)	3	3		2	3					6		4	21
5. decay in shoot and root	25	9	1	1	5		21	15	32	5	3	9	126
6. primary root showing browning, no branching or secondary development shoot usually appearing weak		4	1	1					6		2		14
7. coiling or twisted shoot (shoot appearing weak and showing browning)					1								1
8. spindly, pale or watery shoot			1			5	18		7		1		32
9. weak, spindly primary root		1			1					1			3
total	29	23	3	4	10	5	53	16	57	13	7	13	233
%	7.2	6.0	0.8	1.0	2.5	1.2	13.2	4.0	14.2	3.2	1.8	3.2	

TABLE 4. Fungi associated with the different categories of abnormal seedlings.

Category of abnormal seedlings	Total of abnormal seedlings encountered	Fungi associated
1. no root(s)	5	Pyricularia oryzae
2. no root, decay in shoot	30	Drechslera oryzae
		Fusarium dimerum
		Gibberella zeae
		Pyricularia oryzae
		Trichoconis padwickii
3. short root	1	none
4. decay in shoot (shoot usually appearing weak and showing decay near the point of attachment to the grain)	21	Drechslera oryzae
		D. sorokiniana
		Fusarium dimerum
		F. semitectum
		Gibberella zeae
		Pyricularia oryzae
		Trichoconis padwickii
5. decay in shoot and root	126	Drechslera oryzae
		Fusarium dimerum
		F. longipes
		Gibberella zeae
		Pyricularia oryzae
		Trichoconis padwickii
6. primary root showing browning, no branching or secondary development, shoot usually appearing weak	14	Drechslera oryzae
		Gibberella zeae
		Pyricularia oryzae
		Trichoconis padwickii
7. coiling or twisted shoot (shoot appearing weak and showing browning)	1	Drechslera oryzae
8. spindly, pale or watery shoot	32	Drechslera oryzae
		Trichoconis padwickii
9. weak, spindly primary root	3	none

blotter papers and incubated under NUV light for inducing sporulation. In a few cases *Trichoconis padwickii* was also seen growing along with *Drechslera oryzae* after incubation.

Trichoconis padwickii Ganguly

The fungus was seen on different kinds of abnormal seedlings (Table 4). The growth of the fungus in many cases extended to the entire surface of the shoot. All such seedlings were ultimately killed. Some abnormal seedlings which had no fungal growth, when transferred to the top of blotters, developed abundant characteristic mycelium and fusiform conidia of *T. padwickii* within a few days of incubation.

The fungus also appeared on seedlings having spindly, watery shoots.

Pyricularia oryzae Cav.

This fungus was generally found sporulating at the embryonal end, particularly when seedlings had no root or had only a poorly developed, decayed root-system (Table 4). In seedlings where *P. oryzae* grew at the base of the shoot, the latter appeared weak. In some seedlings having an extensive decay of the root and the shoot, the mycelium and spores of the fungus were seen covering entire seedlings.

Gibberella zeae (Schw.) Petch.

Dark perithecia of *Gibberella zeae* were present on seedlings having decay in the shoot, or on seedlings having browning in the primary root with no branching or no secondary development (Table 4). Only in one seedling pionnotes of the conidial state *Fusarium graminearum* Schwabe were present at the base of the shoot. The fungal growth was restricted to the decayed portions of the shoot without formation of any perithecia on the seed coat.

Discussion

The primary aim of the reported investigations was to examine the pathological aspect concerning abnormal seedlings in rice which are commonly observed during the germination test. Attention was centred on different pathogenic fungi found associated with abnormal seedlings. Nine abnormalitiés categories were observed by the rolled towel method (BP). *Drechslera oryzae* was found associated with about 60%, *Trichoconis padwickii* with 23%, *Gibberella zeae* with 8% and *Pyricularia oryzae* with 5% of the abnormal seedlings inves-

tigated. No fungus was detected in 2% of all abnormal seedlings. These 2% included the seedlings with spindly primary root without secondary development, seedlings with short root and normal shoot, and those without root. There is no information available in the literature about the causes of these abnormalities.

In most of the rice growing countries there are at present no established tolerances for seed-borne pathogens in rice. Obviously, this is because of the fact that routine seed health testing is generally ignored, although germination tests are becoming regular. If the yardstick of a seed testing station is to be applied to the present samples, evidently three samples (number 1083, 1549 and 1592 – Table 1) out of a total of twelve would be rejected for export or even for local sowings because the germination potential was below 80%. On the other hand, ten samples were infected with well known important seed-borne pathogens such as *Drechslera oryzae*, *Pyricularia oryzae*, *Gibberella zeae*, and *Trichoconis padwickii* to such an extent that they would be expected to cause serious crop damage. If a tolerance of 10% for the two first mentioned pathogens for each or in combination is adopted (Table 2), in accordance with the tolerance traditionally adopted in various countries for other leaf spot and leaf blight fungi on cereals and other crops (NEERGAARD, 1962), then ten out of the twelve samples would be rejected. The two remaining accepted samples, No. 1044 and 1546, were not entirely free from infection, but the infection percentages encountered were below the suggested level of tolerance. In principle the tolerance for important pathogens, as those considered here, should be fixed as low as zero. In practice, however, if all available seed samples are more or less infected, one may have to accept seed lots with lower levels of infection.

The standard blotter method with near ultraviolet light permits better growth and sporulation of many fungi. This method gave a vivid picture pertaining to the health condition of the seed samples. During pilot experimentation it was realised that the level of moisture in the blotters during the course of the test was not optimal for germination and seedling evaluation. That is the main reason why the rolled towel method (BP) was conducted for recording germination. It appears that the above mentioned two testing procedures *per se* cannot be combined into a single test; both have to be used in order to assess adequately the planting value of seed lots.

It should be emphasised that seed testing stations should consider the health condition of seed before issuing certificates. Samples showing high germination but infected with important pathogens should be rejected.

A reference should also be made to the fact that at least some of the so called

'normal seedlings' are not *sensu stricto* normal as these may occasionally harbour dangerous pathogens. During the course of this investigation a number of normal seedlings counted on the eighth day in the rolled towel, were picked up at random and examined under a stereobinocular microscope. Some of these were found to carry *Pyricularia oryzae* and *Drechslera oryzae*. Such 'normal' seedlings may become 'abnormal' through rotting if retained beyond the normal standard test period.

Summary

Twelve rice seed samples were tested. The standard rolled towel method was used for seedling evaluation and the blotter method for evaluation of seed-borne fungi. Nine categories of seedling abnormality were recorded. Seedlings with decay in shoot and root were encountered most frequently. *Drechslera oryzae*, *Trichoconis padwickii*, *Gibberella zeae* and *Pyricularia oryzae* were found associated with 60%, 23%, 8% and 5% of the abnormal seedlings. Only in 2% of the abnormal seedlings no fungus was observed. *D. oryzae* was present in 78% of the seedlings with decay in shoot and root, followed by *T. padwickii* (15%) and *G. zeae* (8%). *P. oryzae* occurred in only two abnormal seedlings.

Under existing conditions it was found necessary to run separate tests, one for germination and one for health. Out of the twelve samples investigated, nine would be accepted by a seed analyst based on germination test. However, only two were found acceptable according to criteria based on traditional tolerances in seed health tests of cereals. Even the accepted samples carried pathogens, though at a lower level. Some of the so called 'normal' seedlings in a germination test were found to harbour *D. oryzae* and *P. oryzae*. This further indicates the importance of health testing.

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